APPLICATION OF VARIOUS MATHEMATICAL MODELS TO DATA FROM THE UPTAKE OF METHYL MERCURY IN FISH[1]

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ABSTRACT

This paper evaluates various mathematical models and compares the results of their application to 30-day uptake data from the direct uptake of methyl mercury from water in fish at 9°C and at a CH_3HgCl concentration of 0.2 ppb. The models considered are: (1) linear uptake relationship; (2) first-order kinetics, one-compartment or single-exponential model; (3) combination of first-order kinetics and linear, designated as slope-exponential model; (4) two-compartment or double-exponential model; (5) three-compartment model, including a storage compartment for the build up of contaminant, designated as storage model; (6) three-compartment model, allowing two routes of elimination, designated the "Y"-model; (7) an empirical uptake relationship having the form of a rectangular hyperbola. Although the worst fit of the models was given by the straight line and the best fit by the "Y"-model, the empirical model gave one of the smaller values of the residual mean square. In addition, a comparison of the nonlinear models showed that the empirical model had fewer convergence problems in obtaining parameter estimates and its parameter values had less variation.

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INTRODUCTION

The desire to develop predictive capabilities of environmental phenomena has created a need to characterize many types of observations and interactions. Mathematical modeling has been used to describe the time versus uptake relationship of mercury in aquatic organisms. This paper evaluates various mathematical models and compares the results of their application to 30-day uptake data from the direct uptake of methyl mercury in bluegill sunfish, *Lepomis macrochirus*, at 9°C and at a CH_3HgCl concentration of 0.2 ppb. A discussion is given of the method used to obtain the parameter estimates in each of the models. Radioisotope techniques were used to measure ^{203}Hg-tagged-CH_3HgCl uptake *in vivo* by successive live counts of the inferred methyl mercury body burden in the fish.

The goal of the mathematical models was to describe changes in methyl mercury uptake with time using data obtained from 17 different combinations of temperature and methyl mercury concentrations (Curtis, 1973). The equations do not explicitly treat all of the physical and chemical processes that may be involved; rather they attempt to characterize the observed data. Thus, methyl mercury uptake by fish can be compared statistically and evaluated for trends. These relationships can then be used to predict CH_3HgCl uptake at different temperatures and at different methyl mercury concentrations.

UPTAKE MODELS

Johnels and Westermark (1969) indicated that mercury uptake in fish increased linearly at a given concentration until death occurs, and Scott and Armstrong (1972) maintained that a positive correlation existed between mercury concentration and fish length. Similarly, Kerfoot and White (1972) studied striped bass (Morone saxatilis) ranging up to 12 years of age and found that the mercury content of the axial muscle exhibited an annual increase of 0.059 g of mercury per g of wet weight tissue. These observations indicate the uptake relationship may be a straight line or:

$$Q(t) = a + bt \tag{1}$$

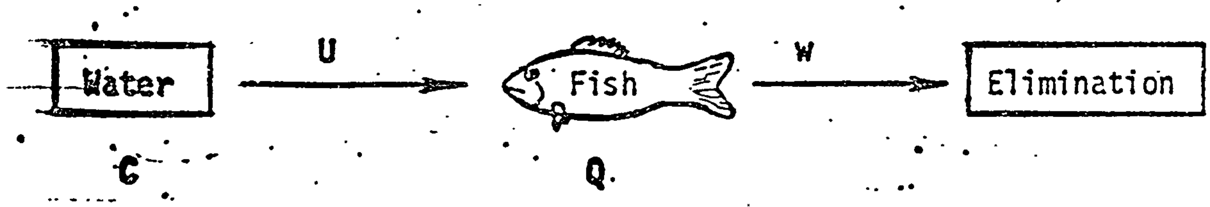
where

$Q(t)$ = contaminant concentration in ppb at time t,

a = intercept value in ppb, and

b = the slope in ppb/hr.

Mellinger (1972), however, applied first-order kinetics to the uptake of $CH_3{}^{203}HgCl$ and $^{203}Hg(NO_3)_2$ in freshwater mussels, (Margaritifera margaritifera) and used a one-compartment or single-exponential model. The accompanying equation gives the mean chemical concentration of ppb in the fish, Q, after time, t, in hours:



$$Q(t) = (UC/w)\ (1 - e^{-wt}) \tag{2}$$

where

U = weight of water that passes over the gills of the fish per weight of the fish per hour or the fraction per hour of chemical absorbed by a fish from the water that passes over its gills,

C = concentration of chemical in the water in ppb, and

w = effective elimination rate of the chemical per hour from the fish, and Q(0) is assumed to be zero.

An alternate version of Eq. (2) is:

$$Q(t) = A + be^{-kt} \tag{3}$$

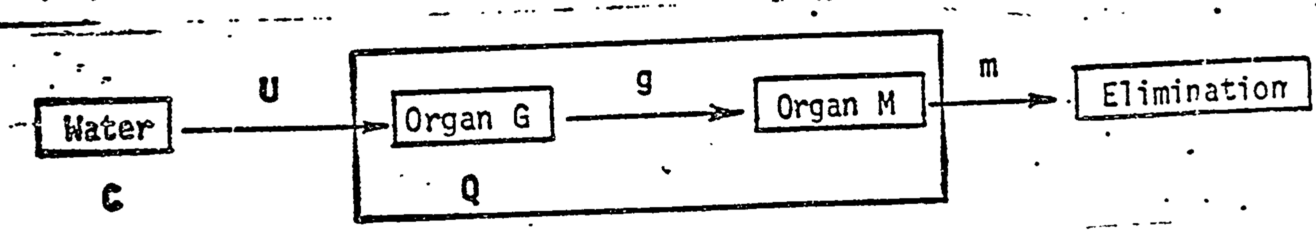
In this form the units for the parameters a and b are in ppb and the units for k are hour^{-1}.

A third model may be obtained by combining first-order kinetics and a sloping line. This model, designated the slope-exponential model, has a sloping line asymptote instead of the maximum value asymptote of the first-order kinetics model. Combining Eqs. (1) and (2) gives:

$$Q(t) = [(UC/w) + bt]\ \ (1 - e^{-wt}) \tag{4}$$

The units are the same as in Eqs. (1) and (2). Without boundaries on the parameters of Eq. (4), this model sometimes behaved like a parabola [that is, the curve increased up to a maximum value and then decreased.] Such a result with a negative estimate of b was contrary to collected data and was unacceptable. With boundaries on the parameters the modeled result often approached the equation of first-order kinetics.

Rucker and Amend (1969) reported that, from a 1-hr exposure, ethyl mercury phosphate was taken up in 8-in rainbow trout (<u>Salmo gairdneri</u>) organs in the following order: gill, blood and muscle (simultaneously), liver, and finally kidney. The incurred ethyl mercury burden was very slowly eliminated from the organs in the same sequential order. This sequence of movement through organs suggests a compartmental model. The sequential movement of a chemical through the two-compartment system and its concentration in the second compartment, M, as a function of time is:



$$M(t) = \frac{UC}{m}(1 - e^{-mt}) + \frac{UC}{m - g}(e^{-mt} - e^{-gt}) \qquad (5)$$

where

C = concentration of the chemical in the water in ppb,

G(t) = chemical concentration in ppb in compartment G at time t,

M(t) = chemical concentration in ppb in compartment M at time t,

Q(t) = whole body concentration of the chemical in ppb at time t,

g and m = turnover rates of the chemical from the respective compartments G and M in units of per hour, and

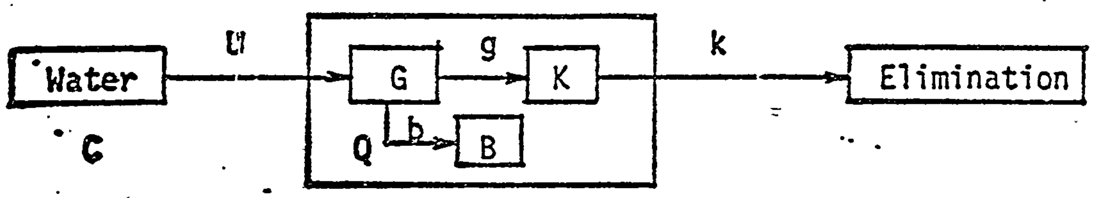
U = uptake of the chemical from the water in units of weight of water that passes over the gills of the fish per weight of that fish per hour.

The expression for the chemical concentration in G is similar to Eq. (2). Summing G and M gives the following expression for whole body data, Q:

$$Q(t) = UC[\frac{1}{g} + \frac{1}{m} + (\frac{1}{g-m} - \frac{1}{g})e^{-gt} - (\frac{1}{m} + \frac{1}{g-m})e^{-mt}] \qquad (6)$$

For this equation and other equations as well, it is assumed that the values for the transfer rates (shown in diagrams) can be determined, these rates are relatively constant, and Q(0), G(0), and M(0) are zero.

Similar to the two-compartment or double-exponential model is a three-compartment model having a storage compartment for the build up of a contaminant. This model, designated as the storage model, may be diagrammed as follows:



If it is assumed that Q(0), G(0), B(0), and K(0) are zero and that the transfer rates, as diagrammed, are relatively constant and can be determined, then the whole body concentrations would be:

$$Q(t) = UC\left[\frac{g(g+b+k)}{k(g+b)^2} + \frac{bt}{g+b} - \frac{g\,e^{-kt}}{k(g+b-k)} + \frac{kg\,e^{-(b+g)t}}{(g+b)^2\,(g+b-k)}\right] \qquad (7)$$

where

C = concentration of the chemical in the water in ppb,

$Q(t)$ = whole body concentration of the chemical in ppb at time t,

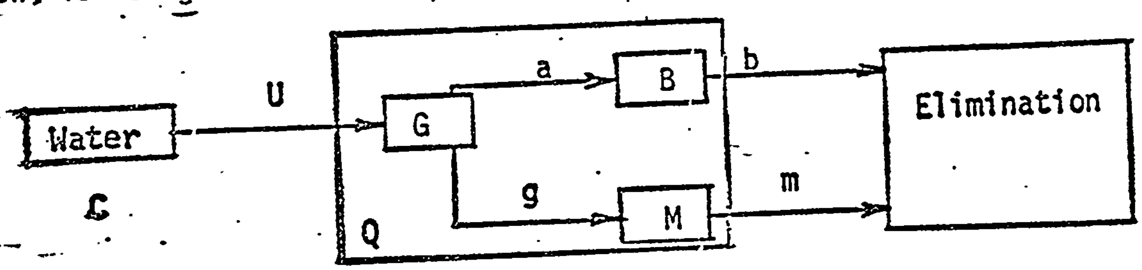
B, G, and K = chemical concentration in ppb in the respective compartments B, G, and K,

b,g and k = turnover rates of the chemical from the respective compartments B, G, and K in units of per hour, and

U = uptake of the chemical from the water in units of weight of water that passes over the gills of the fish per weight of that fish per hour.

The terms of this expression are similar to these of the exponential model in Eq. (4).

An alternate three-compartment model, similar to the storage model, would allow two routes of elimination. This model, diagrammed below, is designated as model Y.



If Q(0), G(0), B(0), and M(0) are zero and the transfer rates, as indicated in the diagram, can be determined and are relatively constant, then the expression describing whole body measurements, Q, is:

$$Q(t) = \frac{UC}{a+g}\left[1 + \frac{a}{b} + \frac{g}{m} + \left(\frac{a}{a+g-b} + \frac{g}{a+g-m} - 1\right)e^{-(a+g)t} - \left(\frac{a}{b} + \frac{a}{a+g-b}\right)e^{-bt} - \left(\frac{g}{m} + \frac{g}{a+g-m}\right)e^{-mt}\right] \quad (8)$$

where

C = concentration of the chemical in the water in ppb,

$Q(t)$ = whole body concentration of the chemical in ppb at time t,

G, B and M = chemical concentrations in ppb in the respective compartments G, B and M,

a and g = turnover rates of the chemical from compartment G in units of per hour,

b and m = turnover rates of the chemical from the respective compartments B and M in units of per hour, and

U = uptake of the chemical from the water in units of weight of water that passes over the gills of the fish per weight of that fish per hour.

If we let $a = g$, $g = b$, and $b = k$ in equation (8), this equation reduces to equation (7) in the limit as m tends to zero.

EMPIRICAL UPTAKE RELATIONSHIP

The various exponential models did not perform adequately when used. Numerical and computer computational difficulties were encountered in obtaining least-squares estimates of the unknown parameters. In the search for an adequate model, an empirical relationship having the form of a rectangular hyperbola was tried and found to be responsive. The empirical relationship, given in Fig. 1, has had the following desirable features:

1. Parameter estimates were less variable with smaller residuals for fish under similar treatments than were the estimates

from other models. This resulted in parameters that could be statistically analysed.

2. The model could simulate straight-line uptake when the data indicated linear uptake.
3. The empirical relationship is simple in form, and the parameters are sensitive to data changes.
4. Numerical and computational difficulties were not encountered.
5. The parameters obtained for the individual fish compared favorably with the parameters of that group analyzed as a whole.

As an additional comparison, the previously derived models were fit to the same group of data for fish studied at 9°C and 0.2 ppb CH_3HgCl. The weighted residual mean square, defined by

$$\sum_{i=1}^{N} W_i \, [Q_i(\text{observed}) - Q_i(\text{estimated})]^2/(N-p)$$

where W_i is the weight for the ith observation and p is the number of parameters in the model under consideration, was calculated. The results of these calculations are shown in Table 1. Since a smaller value of this weighted residual mean square indicates a better overall fit to the data by a particular model (perfect fit would give a value of zero), it may be seen that the worst fit was given by the straight line. Although the smallest value was given by the "Y" model, the empirical model gave one of the smaller values.

The desirable features of the empirical relationship led to its use in describing the time versus methyl mercury uptake relationship for each individual fish and for the group of fish at each of 17 study conditions (Curtis, 1973). Parameters from these uptake curves enabled estimates to be made of the CH_3HgCl body burden of each fish at any time. Individual and interactive effect of temperature and concentration of $CH_3{}^{203}HgCl$ uptake were found to be significant at the 0.01 level. Trends in parameter values over changes in temperature and concentration were developed to estimate methyl mercury uptake in fish at a given temperature and methyl mercury concentration (Curtis, 1973).

Each fish was live counted almost daily for the ^{203}Hg gamma emission to obtain each data point. Fig. 2 shows a series of data points from one fish and Fig. 3 shows data for all of the fish studied at 9°C and 0.2 ppb CH_3HgCl. The empirical model was fitted to the data in Figs. 2 and 3 to characterize the ^{203}Hg inferred CH_3HgCl uptake.

Four models, straight-line, single-compartment, empirical, and slope-exponential, were fit to the same group of data and are given in Fig. 4.

The estimated parameter values indicate that the more complicated models, two-compartment and storage, made only slight improvement in describing the observed data. Comparison is made in Fig. 5 for the storage model on one representative fish. Various parameter values are given with the different curves in Fig. 5. This figure indicates that a large change in the exponential parameter values for this model has a negligible effect on the appearance of the uptake curve. For this reason the simpler models in Fig. 4 are of prime interest.

ESTIMATION OF PARAMETERS

An analysis of the variability of the observations at each observation time showed a significant ($P < .01$) increase from earlier to later times of observation. Therefore, a weighted regression analysis was performed in estimating the unknown parameters from the combined data set for all fish. The unknown parameters in Eqs. (1)-(8) and Fig. 1 were obtained by minimizing the expression

$$\sum_{i=1}^{N} W_i[Q_i(\text{observed}) - Q_i(\text{estimated})]^2 \qquad (9)$$

where N is the total number of observations and W_i is the weight for the i<u>th</u> observation. In this data set all of the observations at a particular time of observation received equal weight, which is equal to the reciprocal of the sample variance of the observation at that particular time. It should be noted that the minimization of Eq. (9) reduces to the usual unweighted regression analyses when $W_i = 1$ for all i. Since multiple observations at a particular observation time were not available on an individual fish, unweighted parameter estimates were obtained for individual fish.

When the term Q_i (estimated) in Eq. (9) is nonlinear in the unknown parameters, for example, parameter w in Eq. (2), then an iterative technique used in order to obtain the parameter estimates. The technique used in this analysis involved the expansion of the expression Q_i (estimated) about a point of initial parameter estimates in a Taylor series expansion through the linear terms. Q_1 was substituted into Eq. (9) to obtain a new set of parameter estimates, which takes the place of the initial estimate; the procedure is continued until the parameter values change by less than some specified value from one iteration to another. A detailed discussion of this technique may be found in Draper and Smith (1966).

DISCUSSION AND CONCLUSIONS

Data from the direct uptake of methyl mercury in bluegill from water at 9°C and 0.2 ppb methyl mercury as a function of time were mathematically simulated by several models: (1) straight-line; (2) one-compartment or single-exponential; (3) slope-exponential; (4) two-compartment or double-exponential; (5) three-compartment or storage; and (6) empirical, having the form of a rectangular hyperbola.

Each of the models examined has favorable attributes; however, the empirical model was superior in adapting to the data and describing it in a manner by which predictive estimation procedures could be developed.

Comparison of the results of the various models used to simulate the uptake data showed that the empirical model described the data with a smaller residual variance and that its parameter values, from individual fish and group data, grouped better and had less variation.

A straight line may adequately describe the data over certain short segments of time (<200 hr) but not over long periods of time. The horizontal asymptotic properties of the one-compartment model appear to be unrealistic when comparing these data with long-term data of others. The empirical model also has an ultimate horizontal asymptote; however, its value is greater than that of the one-compartment model and therefore is less limiting.

The multi-compartment models did not appear to be applicable to these data since the parameter values for the additional compartments (after the 1st) varied greatly with only a small effect on the model fit to the data. The slope-exponential model is the derived model which appears to have the greatest promise for describing long-term uptake data. The empirical model is able to adequately describe the data, even though it has an ultimate horizontal asymptote, because its asymptotic value is greater than the upper toxic concentration found in the fish (about 20 ppm) and, at the upper toxic level, the empirical model is still increasing as is the mercury burden in the fish.

REFERENCES

Curtis, E. H., Ph.D. dissertation, Northwestern University, Evanston, Ill., 1973.

Draper, N. R., Smith, H., "Applied regression analysis," pp. 263-304, John Wiley and Sons, Inc., New York, 1966.

Johnels, A. G., Westermark, T., in Miller, M. W., Berg, G. G., Eds., "Chemical Fallout," pp. 221-241, C. C. Thomas, Springfield, Ill., 1969.

Kerfoot, W. B., White, G. G., "Mercury content of modern and old striped bass (Morone saxatilis)," Woods Hole Oceanographic Institution Contribution No. 2778, Woods Hole, Mass., 1972.

Millinger, P. J., "The comparative metabolism of two mercury compounds as environmental contaminants in the freshwater mussel (Margaritifera margaritifera)," pp. 173-180, 6th Ann. Conf. on Trace Substances in Environmental Health, June 13-15, 1972, Columbia, Mo., 1972.

Rucker, R. R., Amend, D. F., Prog. Fish-Cult., 31, 4, 197-201, 1969.

Scott, D. P., Armstrong, F. A. J., J. Fish. Res. Bd. Can., 29, 1085-1090, 1972.

FIGURE LEGENDS

Figure 1. Empirical uptake relationship in the form of a rectangular hyperbola used to describe the uptake of methyl mercury by fish.

Figure 2. Modeled data from the direct uptake of CH_3HgCl in a fasted bluegill from water at 9°C and 0.2 ppb CH_3HgCl. An empirical model having the form of a rectangular hyperbola has been fit to the data. Water temperature and CH_3HgCl concentration are also plotted.

Figure 3. The direct uptake of CH_3HgCl in fasted bluegill from water at 9°C and 0.2 ppb CH_3HgCl. An empirical model having the form of a rectangular hyperbola has been fit to the data.

Figure 4. Comparison of models to data from the direct uptake of CH_3HgCl in fasted bluegill from water at 9°C and 0.2 ppb CH_3HgCl. (a) Straight line $Y(t) + A+Bt$; (b) Rectangular hyperbola $Y(t) = A+t/(B+Ct)$; (c) Single exponential $Y(t) = (CU/W)\ (1-e^{-Wt})$; (d) Slope exponential $Y(t) = (CU/W + Bt)\ (1-e^{-Wt})$.

Figure 5. Effect of different parameter values on the equation describing the storage model.

Table I. Comparison of Models From Weighted Residual Mean Square

Model	Equation #	Residual Mean Square
Linear	1	1.666
Single Compartment	2	1.143
Slope Exponential	4	1.007
Two Compartment	6	1.148
Storage	7	1.004
"Y"	8	0.999
Rectangular Hyperbola	Figure 1	1.024

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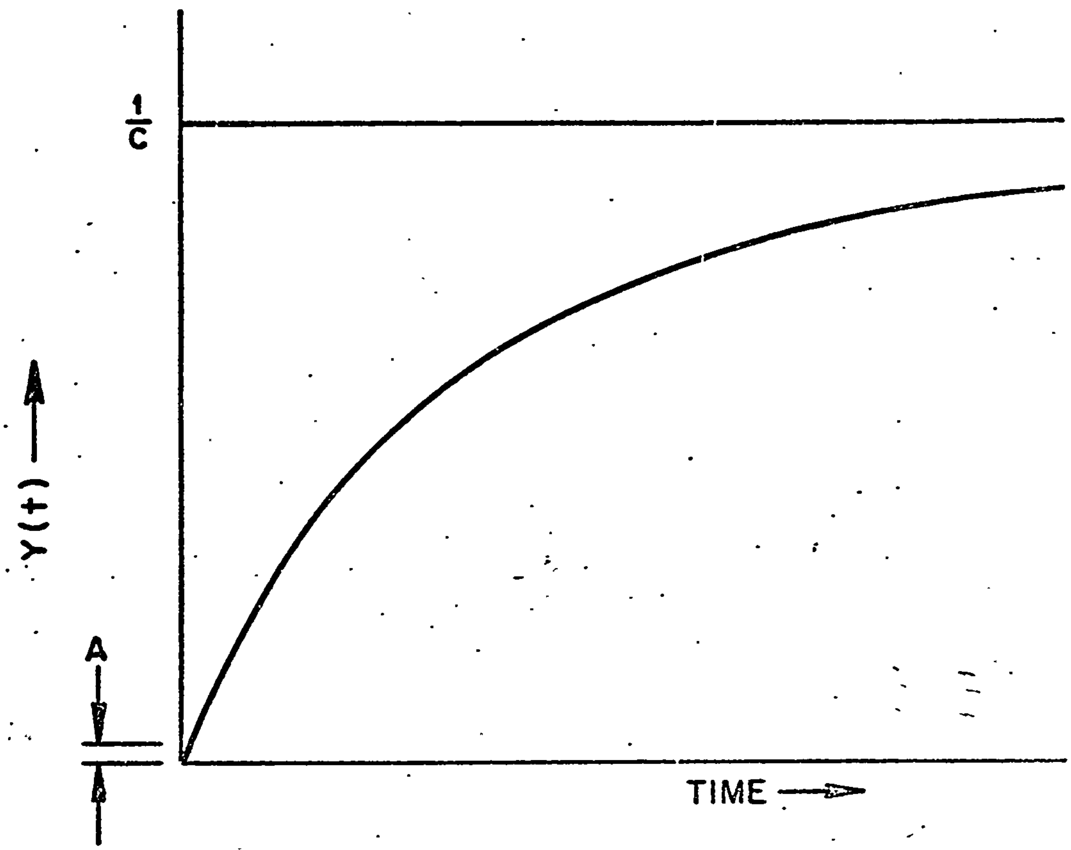


$$Y(t) = A + \frac{t}{B + Ct}$$

where:

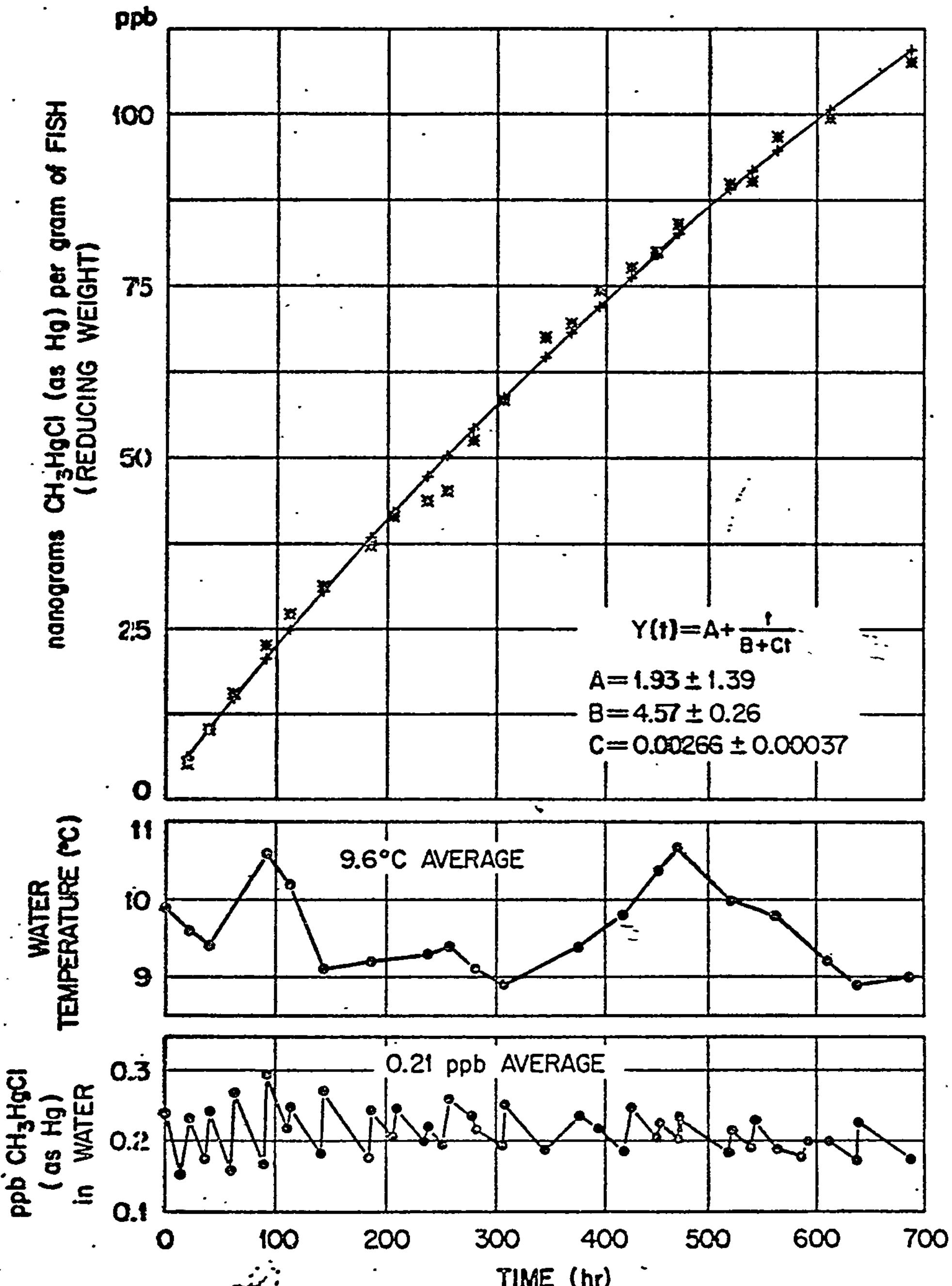
A = Y axis intercept

B, describes the slope or uptake rate (the smaller B, the greater the slope)

C, describes the curvature of the data plot and the asymptotic value (the closer C approaches zero the straighter the curve and the greater the asymptotic value)

t = Time; as $t \rightarrow \infty$, $Y(t) \rightarrow A + 1/C$

Empirical Uptake Relationship in the Form of a Rectangular Hyperbola.



Modeled Data From the Direct Uptake of CH_3HgCl in a Fasted Bluegill From Water at 9°C and 0.2 ppb CH_3HgCl.

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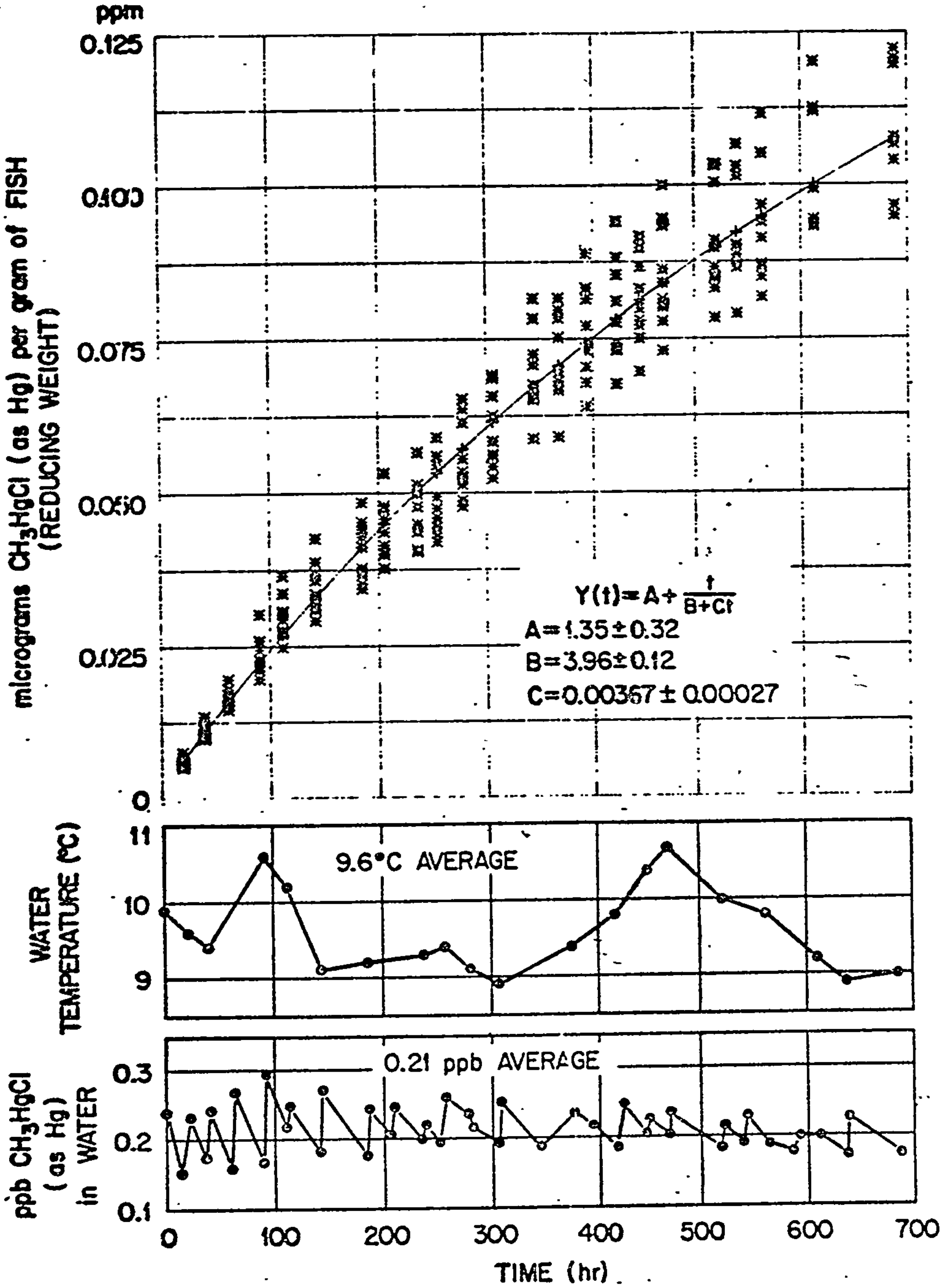
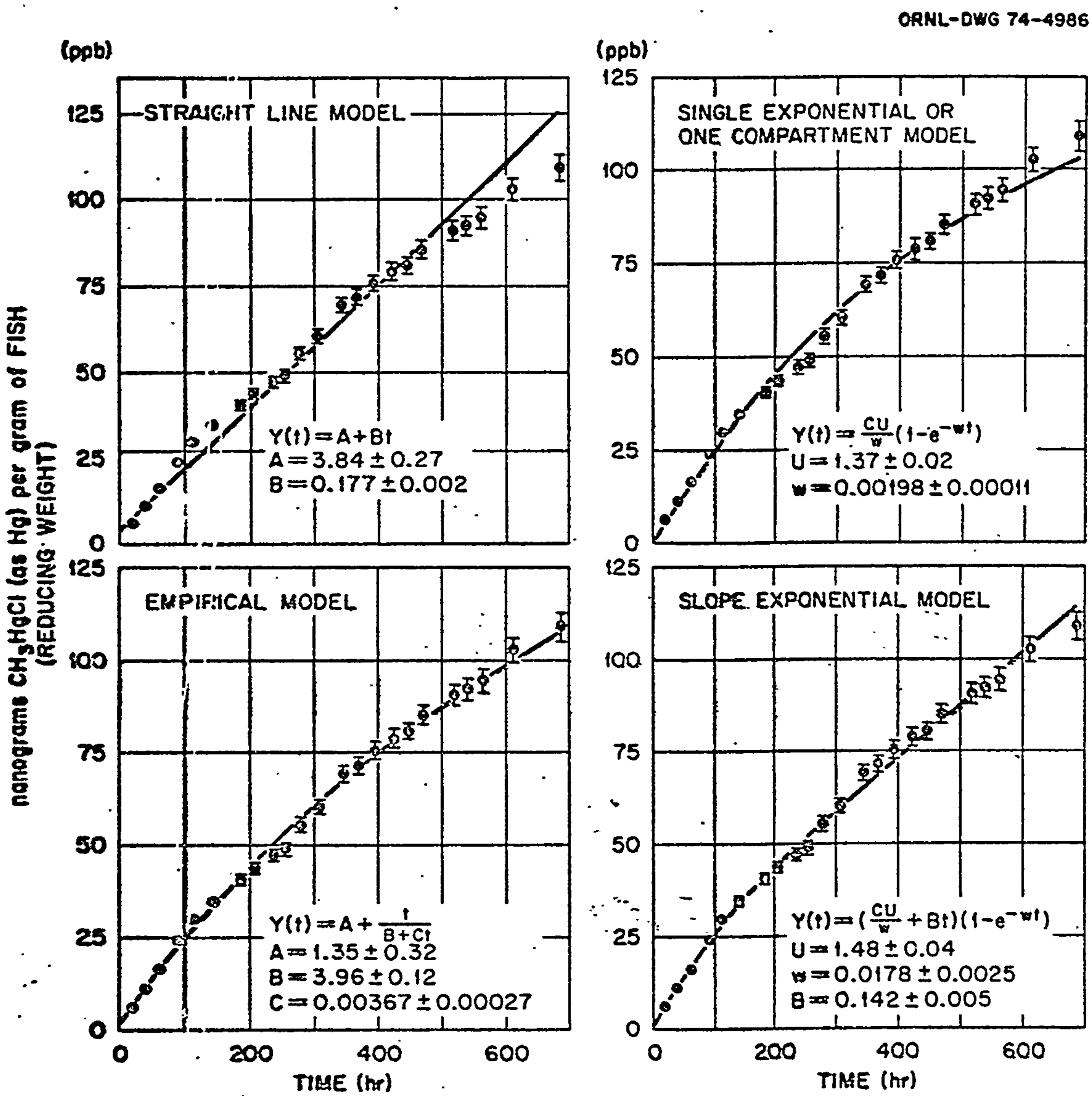
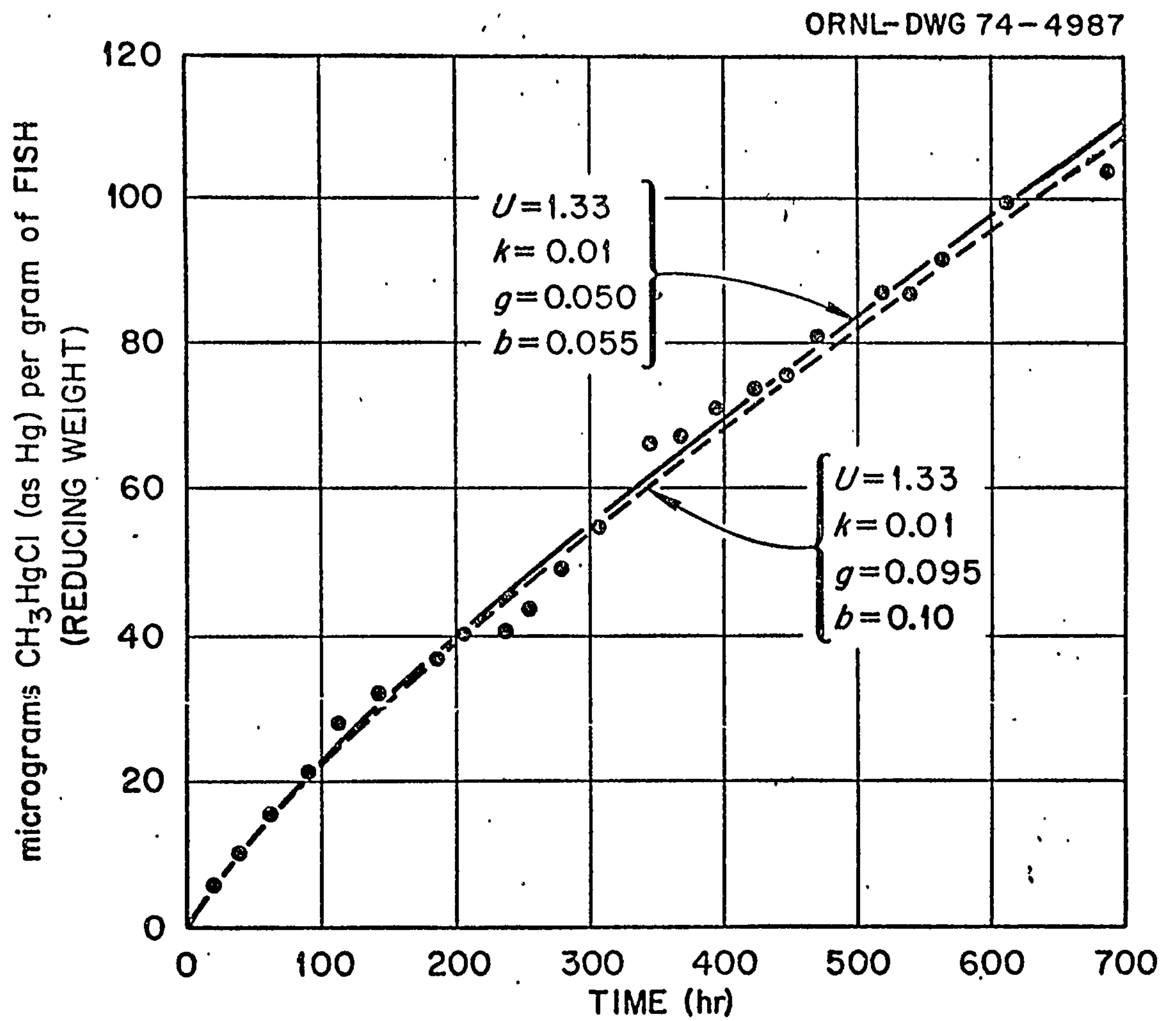


The Direct Uptake of CH_3HgCl in Fasted Bluegill From Water at 9°C and 0.2 ppb CH_3HgCl.



Comparison of Models to Data from the Direct Uptake of CH_3HgCl in Fasted Bluegill from Water at 9°C and 0.2 ppb CH_3HgCl.



Effect of Different Parameter Values on the Storage Model.